A Novel Force Discrimination Assay Using Magnetic Beads

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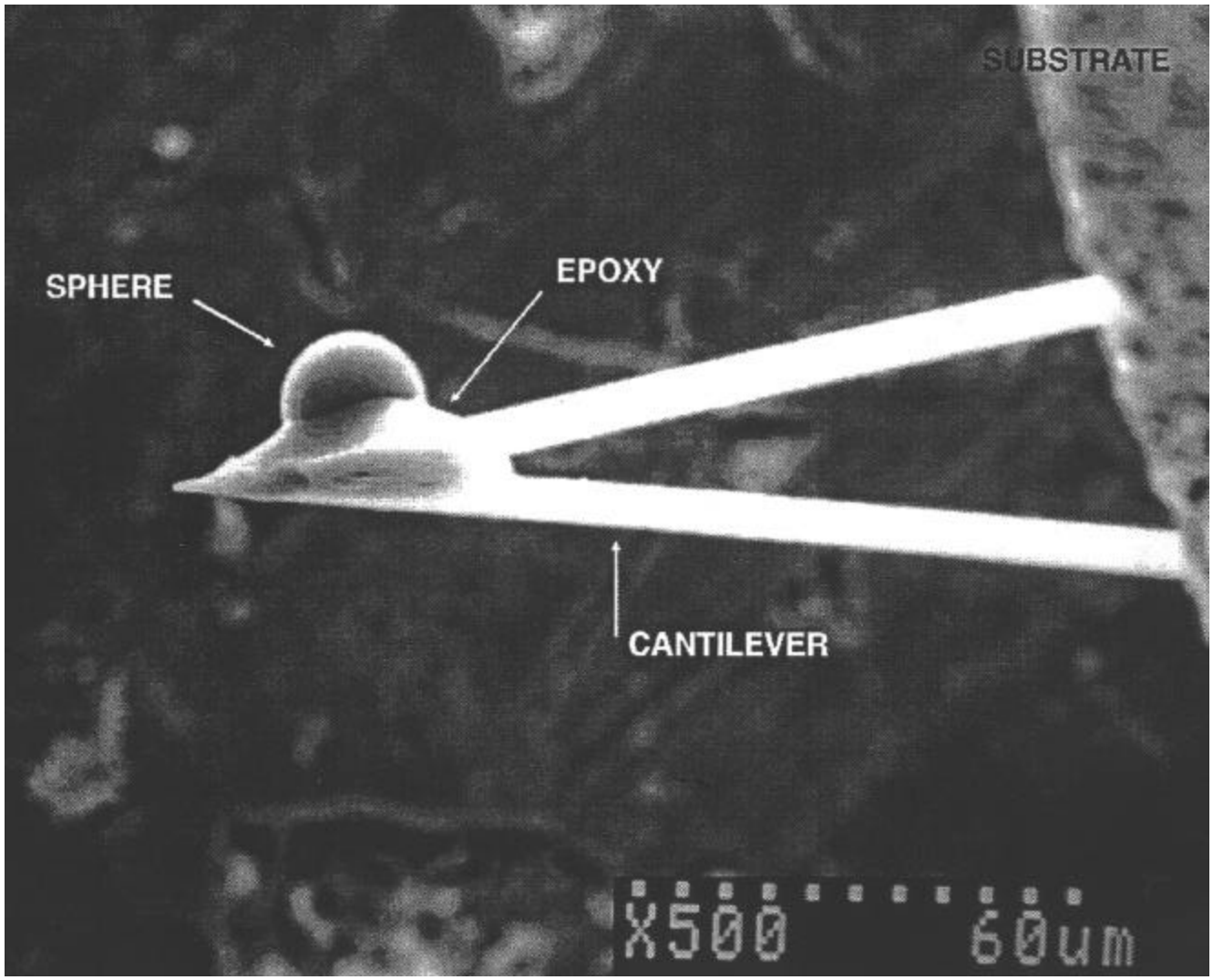
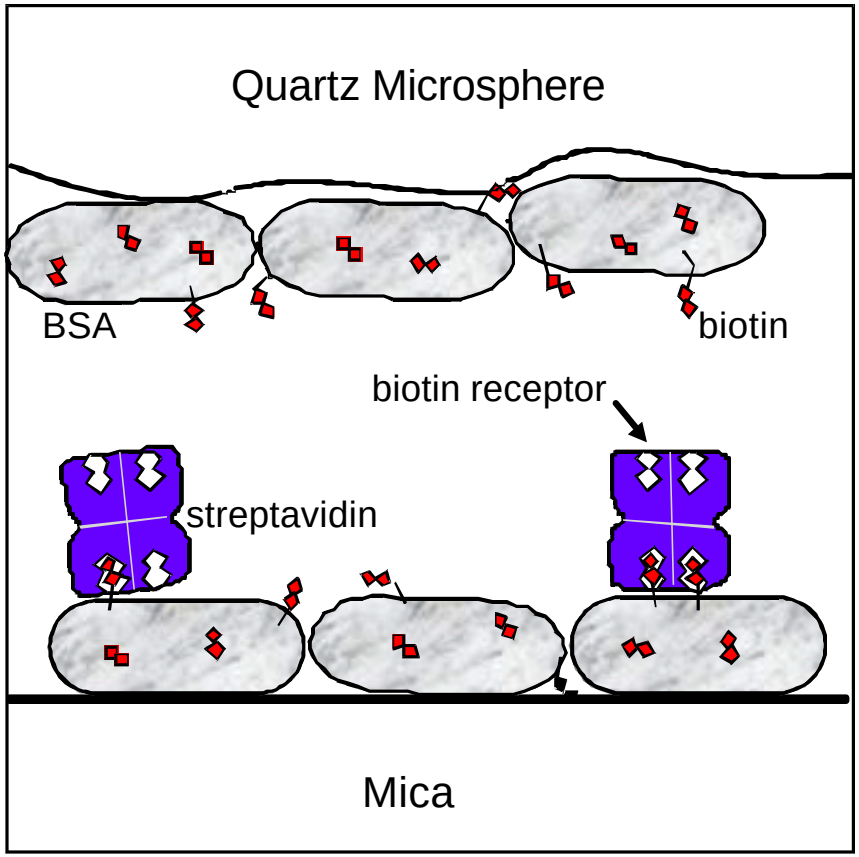
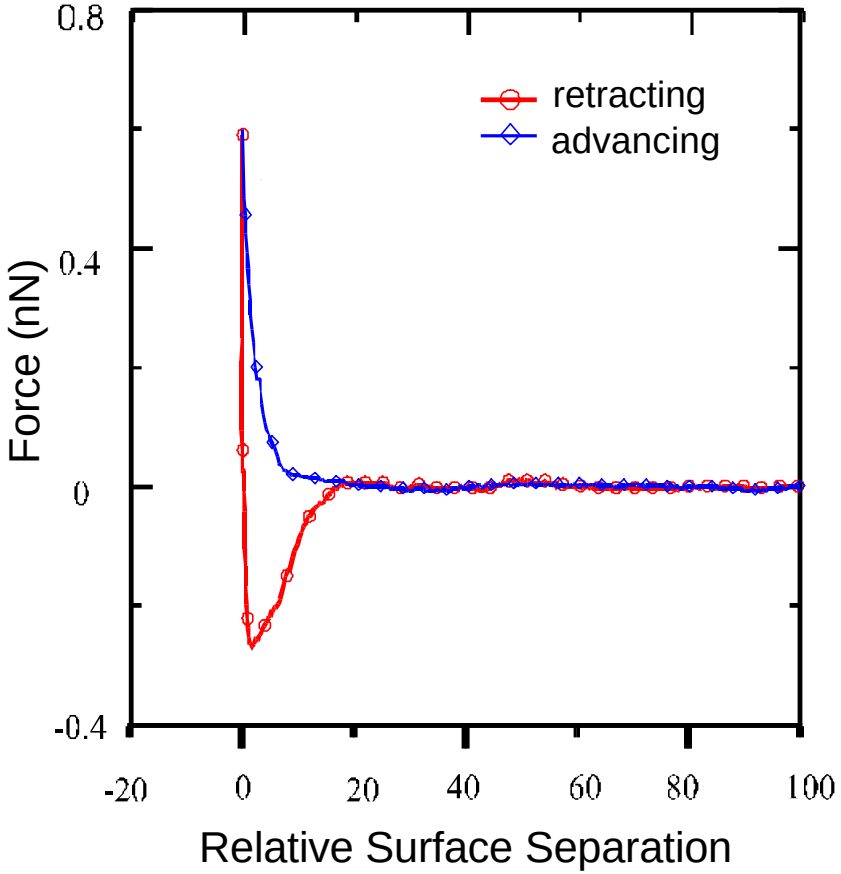
Quartz Sphere on AFM Cantilever



Using AFM to Measure Forces Between Single Molecules





G.U Lee, D.A. Kidwell & R.J. Colton, *Langmuir* **10**, 354 (1994).

Molecular Recognition & Force Discrimination

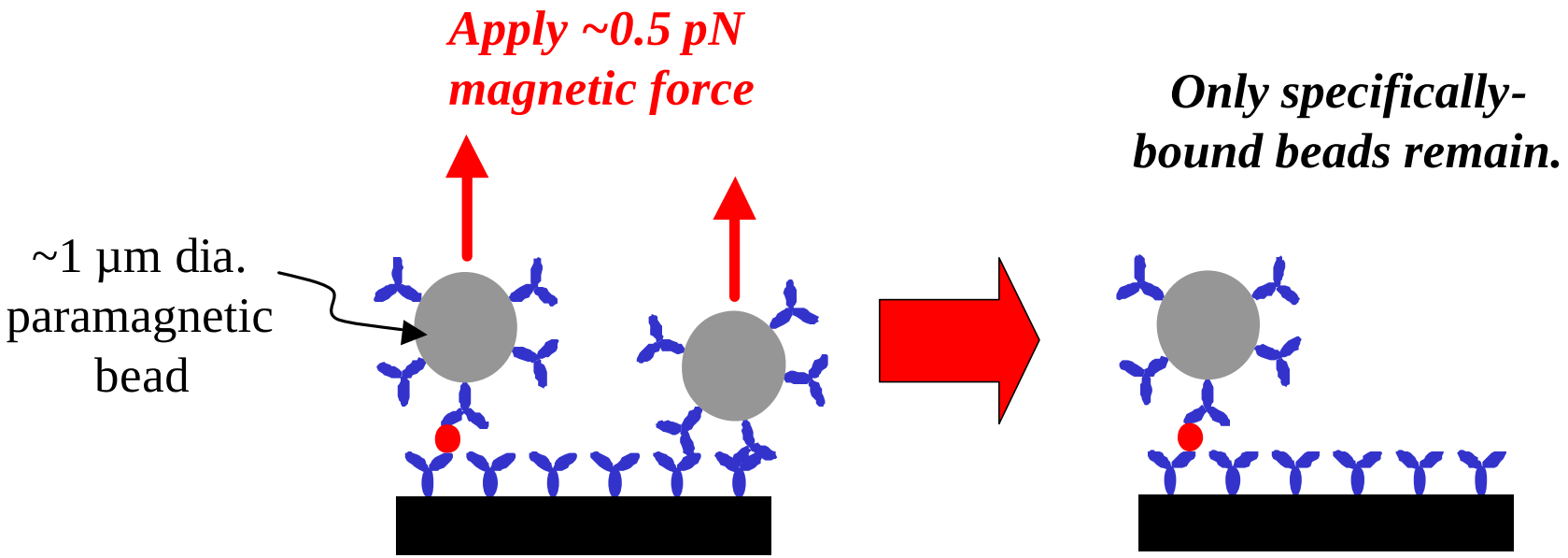
- Range of measured rupture force

DNA	(20 mer in 100 mM NaCl)	750 +/- 120 pN
	(20 mer in 10 mM NaCl)	550 +/- 70 pN
Streptavidin-Biotin		200 - 300 pN
Antigen-Antibody		40 pN

- If force discrimination is implemented in a biosensor we anticipate **increased**
  - selectivity (fewer false positives and negatives)
  - sensitivity (lower background)

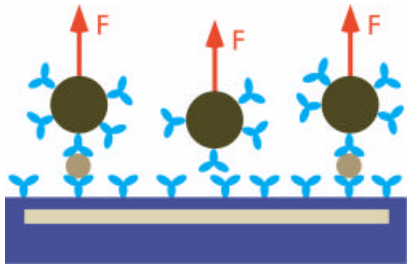
Immunobead Force Discrimination Assay

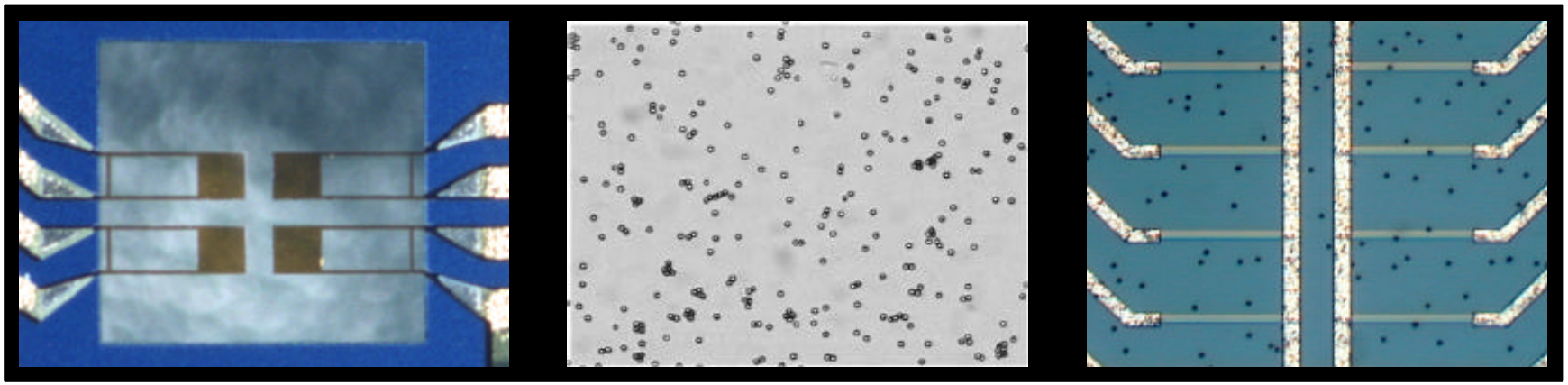
- Use paramagnetic beads as labels
- Magnetic field gradient removes nonspecifically bound beads
- ***Magnetic force eliminates background***



Revolutionary Biosensor Technology

Three Detection Methods





Piezoresistive cantilever
FABS

D.R. Baselt, *et al.*, *Proc. IEEE* **85**, 672 (1997)

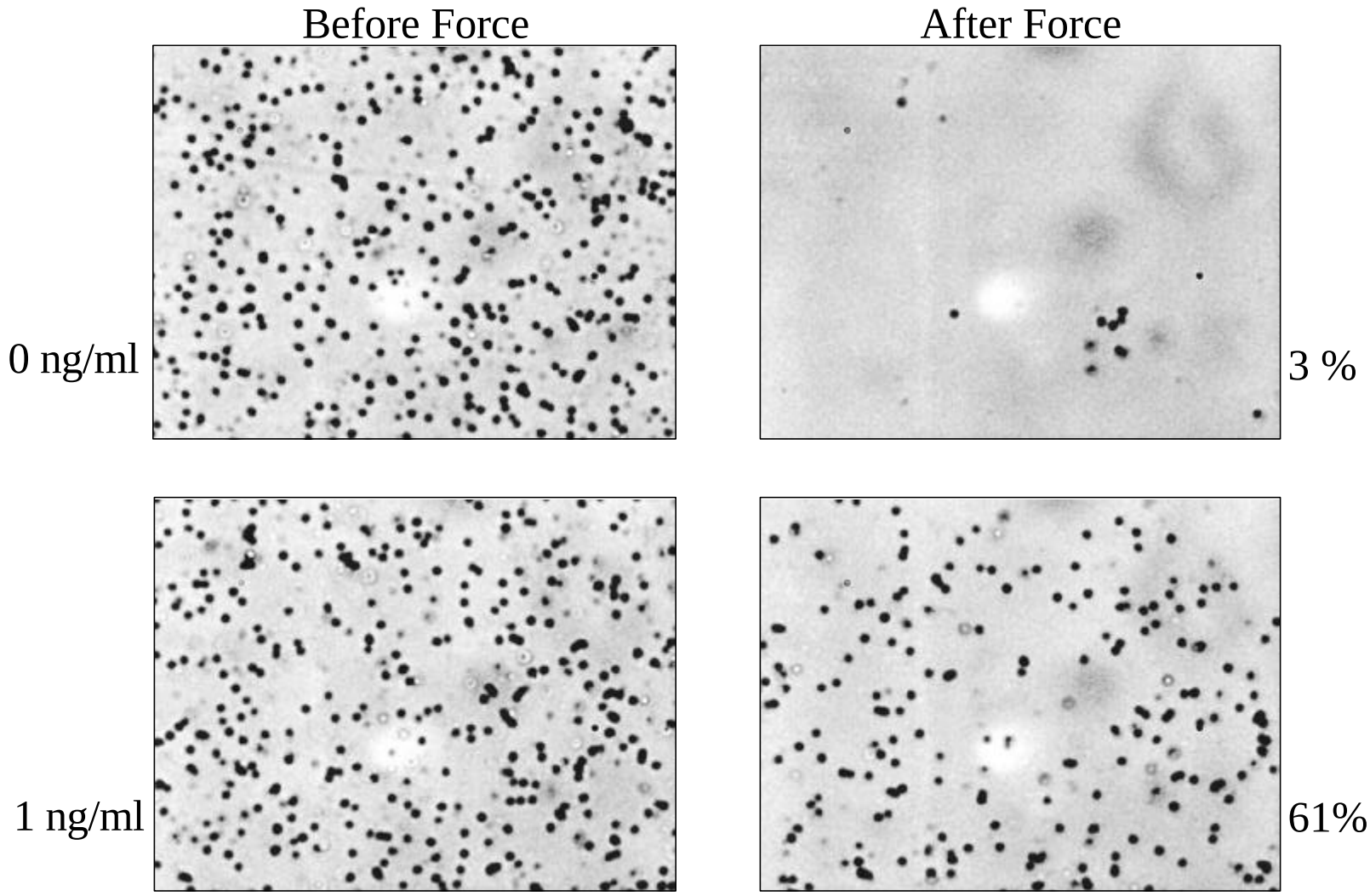
Transparent substrate & optical microscope
FDB

G.U. Lee, *et al.*, *Anal. Biochem*. **287**, 261 (2000)

Magnetoresistive elements
BARC

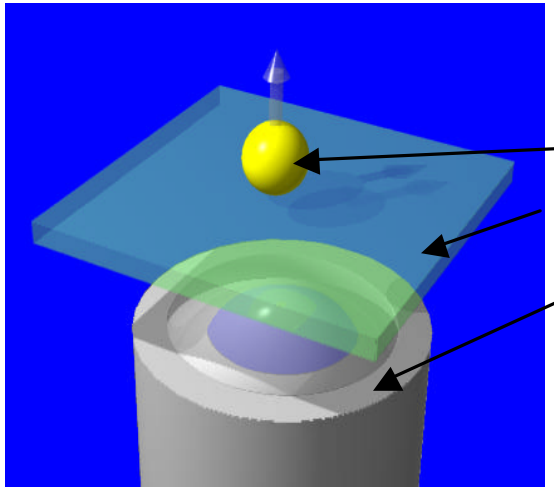
R.L. Edelstein, *et al.*, *Biosensors & Bioelectronics* **14**, 805 (2000)

FDB: Ovalbumin Detection on Transparent Substrates



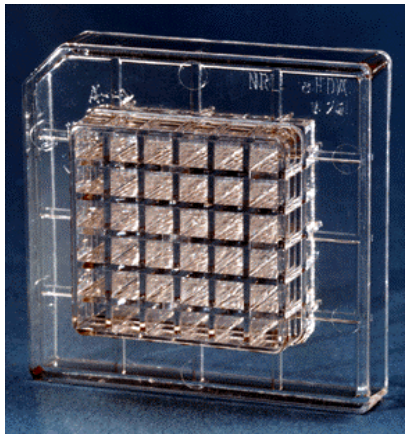
G. Lee, *et al.*, *Analytical Biochemistry* **287,** 261 (2000)

Optical-based Force Discrimination Biosensor

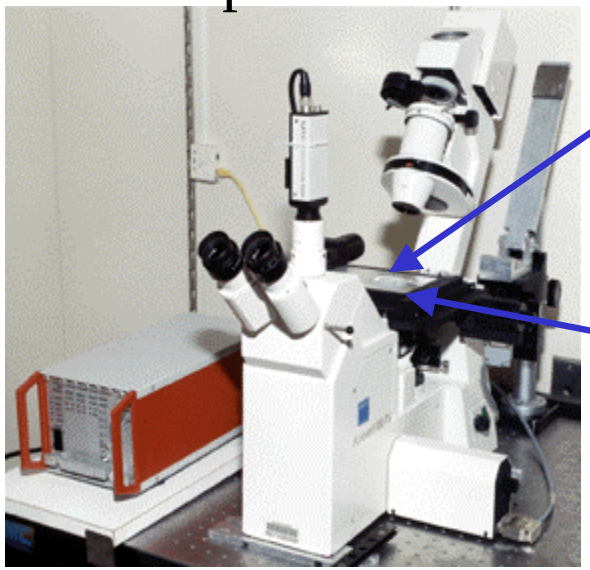


magnetic bead
transparent substrate
optical microscope

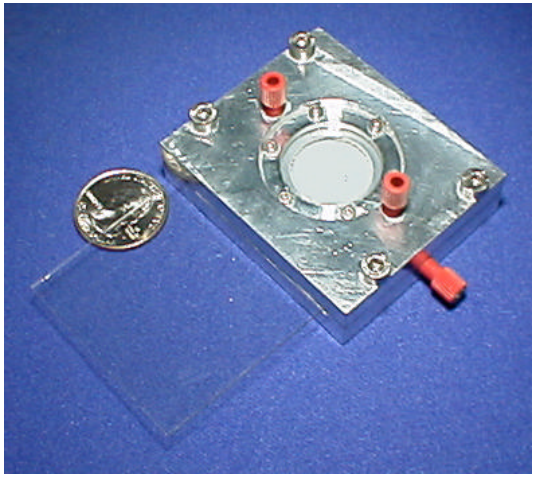
Polystyrene microtiter well



Inverted optical microscope



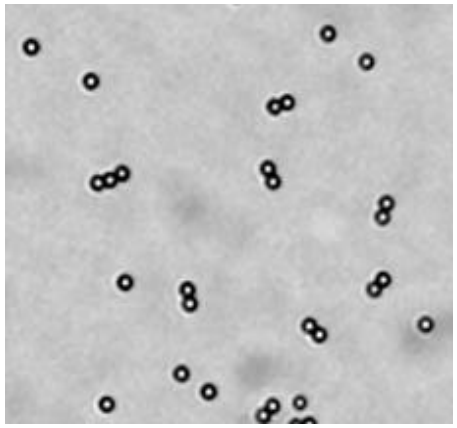
Ultrafiltration membrane



Immunobead Force Discrimination Assay

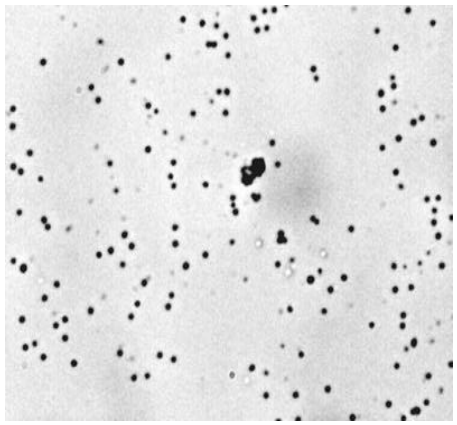
Magnetic Beads

PEI-PEG-Antibody Chemistry



Dynal, 2.8 μm size beads containing, polymer coated mono-dispersed magnetic material (Fe_3O_4 and δFe_2O_3)

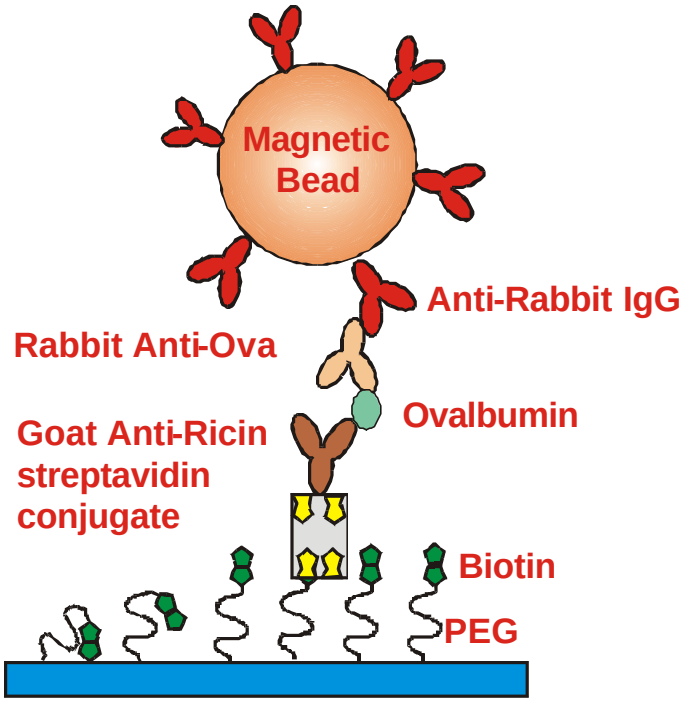
M ~ 12 emu/cm^3

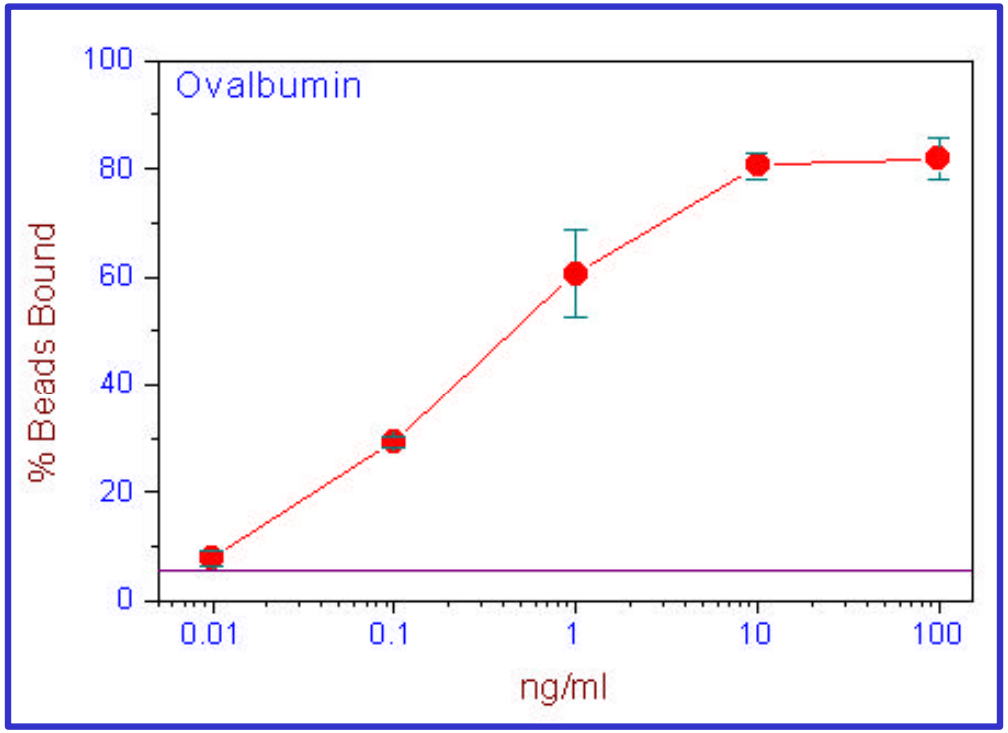


SeraMag, 0.8 μm size beads containing a magnetite core coated with polymer

M ~ 38 emu/cm^3

Immunobead Force Discrimination Assay
Results for Ovalbumin





- Detection sensitivity = 10 pg/mL
- Assay time = 25 min.

Immunobead Force Discrimination Assay
Conclusions

- Sensitivity of our immunobead force discrimination assay is ~10^3 X higher than conventional immunoassays such as ELISA
- Specificity is typically > 99%
- Response time is comparable to PCR-based instruments
- Better magnetic beads are needed to improve force discrimination between molecules